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PATENT & TRADEMARK OFFICE

ATTACHMENT B
Amendments to the Specification

Please replace the paragraph at page 9, lines 13-18 with the following amended paragraph:

According to the present invention, the "Ulip6/CRMP5 protein" refers to a protein substantially comprising the amino-acid sequence shown in SEQ ID ~~n°~~NO: 2, as well as polypeptide fragments and derivatives thereof. A nucleic acid sequence coding for the Ulip6/CRMP5 protein comprises the nucleic acid sequence from nucleotides 163 to 1854 in SEQ ID ~~n°~~NO: 1, or degenerates thereof.

Please replace the paragraph at page 9, lines 19-24 with the following amended paragraph:

The "Ulip2/CRMP2 protein" refers to a protein substantially comprising the amino-acid sequence shown in SEQ ID ~~n°~~NO: 4 (also available on EMBL/Genbank database under access number U 17279), as well as polypeptide fragments or derivatives thereof. A nucleic acid sequence coding for the Ulip2/CRMP2 protein comprises the nucleic acid sequence from nucleotides 72 to 1790 in SEQ ID ~~n°~~NO: 3, or degenerates thereof.

Please replace the paragraph at page 9, lines 25-30 with the following amended paragraph:

The "Ulip1/CRMP4 protein" refers to a protein substantially comprising the amino-acid sequence shown in SEQ ID ~~n°~~NO: 6 (also available on EMBL/Genbank database under access number Y 07818), as well as polypeptide fragments or derivatives thereof. A nucleic acid sequence coding for the Ulip1/CRMP4 protein comprises the nucleic acid sequence SEQ ID ~~n°~~NO: 5, or degenerates thereof.

Please replace the paragraph at page 9, line 31 through page 10, line 3 with the following amended paragraph:

The "Ulip3/CRMP1 protein" refers to a protein substantially comprising the amino-acid sequence shown in SEQ ID ~~n°~~NO: 8 (also available on EMBL/Genbank database under access number D 78012), as well as polypeptide fragments or derivatives thereof. A nucleic acid sequence coding for the Ulip3/CRMP1 protein comprises the nucleic acid sequence SEQ ID ~~n°~~NO: 7, or degenerates thereof.

Please replace the paragraph at page 10, lines 4-8 with the following amended paragraph:

The "Ulip4/CRMP3 protein" refers to a protein substantially comprising the amino-acid sequence shown in SEQ ID ~~n°~~NO: 10, as well as polypeptide fragments or derivatives thereof. A nucleic acid sequence coding for the Ulip4/CRMP3 protein comprises the nucleic acid sequence SEQ ID ~~n°~~NO: 9, or degenerates thereof.

Please replace the paragraph at page 10, lines 11-17 with the following amended paragraph:

Derivative polypeptide refers to any variant polypeptide of the proteins above or any other molecule resulting from a modification of genetic and/or chemical nature of the sequence SEQ ID ~~n°~~NO: 2, ~~n°~~ SEQ ID NO: 4, ~~n°~~ SEQ ID NO: 6, ~~n°~~ SEQ ID NO: 8, or ~~n°~~ SEQ ID NO: 10, that is to say obtained by mutation, deletion, addition, substitution and/or chemical modification of a single or of a limited number of amino acids, as well as any isoform sequence, the said modified or isoform variant sequences having conserved at least one of the properties making them biologically active.

Please replace the paragraph at page 10, lines 18-23 with the following amended paragraph:

The invention likewise relates to the use of an isolated nucleic acid sequence selected from SEQ ID ~~n°~~NO: 1, ~~n°~~NO: 3, ~~n°~~NO: 5, ~~n°~~NO: 7, or ~~n°~~NO: 9, or a nucleotide fragment or derivative sequences derived from the sequences SEQ ID ~~n°~~NO:

1, ~~n°~~NO: 3, ~~n°~~NO: 5, ~~n°~~NO: 7, or ~~n°~~NO: 9, on account of the degeneracy of the genetic code, or on account of mutation, of deletion or of insertion of at least one nucleotide.

Please replace the paragraph at page 10, line 26 through page 11, line 2 with the following amended paragraph:

The derivative nucleotide sequences also include sequences capable of hybridizing strongly and specifically with SEQ ID ~~n°~~NO: 1, ~~n°~~ SEQ ID NO: 3, ~~n°~~ SEQ ID NO: 5, ~~n°~~ SEQ ID NO: 7, or ~~n°~~ SEQ ID NO: 9 or their complementary sequences. The appropriate hybridization conditions correspond to the conditions of temperature and of ionic strength usually used by the person skilled in the art (Sambrook et al, 1989), preferably to temperature conditions of between T_m minus 5°C and T_m minus 30°C and more preferably to temperature conditions of between T_m minus 5°C and T_m minus 10°C (great stringency), T_m being the theoretical melting point, defined as being the temperature at which 50 % of the paired strands separate.

Please replace the paragraph at page 11, lines 3-6 with the following amended paragraph:

The nucleotide sequences SEQ ID ~~n°~~NO: 1, ~~n°~~NO: 3, ~~n°~~NO: 5, ~~n°~~NO: 7, or ~~n°~~NO: 9 are useful for the production of antisense sequences capable of hybridizing specifically with a nucleic acid sequence, including a messenger RNA, which can be used in gene therapy.

Please replace the paragraph at page 13, line 28 through page 14, line 2 with the following amended paragraph:

In a preferred embodiment, said Ulip/CRMP protein is Ulip6/CRMP5 and/or Ulip2/CRMP2. Preferably, the nucleic acid according to the invention may be a nucleic acid coding for the Ulip6/CRMP5 protein that comprises the nucleic acid sequence from nucleotides 163 to 1854 in SEQ ID ~~n°~~NO: 1, or degenerates thereof. Also preferably, the nucleic acid according to the invention may be a nucleic acid coding

for the Ulip2/CRMP2 protein that comprises the nucleic acid sequence from nucleotides 72 to 1790 in SEQ ID ~~n°~~NO: 3, or degenerates thereof.

Please replace the paragraph at page 15, lines 12-14 with the following amended paragraph:

The invention likewise relates to mono- or polyclonal antibodies directed against Ulip/CRMP polypeptide comprising an amino acid sequence selected from SEQ ID ~~n°~~NO: 2, ~~n°~~ SEQ ID NO: 4, ~~n°~~ SEQ ID NO: 6, ~~n°~~ SEQ ID NO: 8, or ~~n°~~ SEQ ID NO: 10.

Please replace the paragraph at page 16, lines 1-3 with the following amended paragraph:

Thus, the invention also relates to aptamers directed against Ulip/CRMP polypeptide comprising an amino acid sequence selected from SEQ ID ~~n°~~NO: 2, ~~n°~~ SEQ ID NO: 4, ~~n°~~ SEQ ID NO: 6, SEQ ID ~~n°~~NO: 8, or ~~n°~~ SEQ ID NO: 10.

Please replace the paragraph at page 20, lines 11-15 with the following amended paragraph:

The vectors which are particularly preferred for the transfection of mammalian cells are vectors containing the cytomegalovirus (CMV) promoters such as pcDNA1 (Invitrogen), vectors containing the MMTV promoter such as pMAMNeo (Clontech) and pMSG (catalogue ~~n°~~NO 27-4506-01 from Pharmacia) and vectors containing theSV40 promoter such as pSVβ (Clontech).

Please replace the paragraph at page 22, lines 19-29 with the following amended paragraph:

Antibodies. The peptides chosen to generate specific antisera were KEMGTPLADTPTRPVTRHGG (SEQ ID ~~n°~~NO: 11, amino acids 505-524) for anti-Ulip6/CRMP5, LEDGTLHVTEGS (SEQ ID ~~n°~~NO: 12) and ITGPEGHVLSRPEEVE (SEQ ID ~~n°~~NO: 13) (amino acids 454-465 and 217-232, respectively) for anti-Ulip2/CRMP2,

LTSFEKWHEAADTKS (SEQ ID ~~n°~~NO: 14, amino acids 117-131) for anti-Ulip3/CRMP1, and EHDSHAQLRWRVL (SEQ ID ~~n°~~NO:15, amino acids 664-676) for anti-neuropilin-1. The synthetic peptides were conjugated to keyhole limpet hemocyanin and used to immunize rabbits or rats as previously described (Honnorat et al., 1999). The antibodies were purified from anti-Ulip6/CRMP5, anti-Ulip3/CRMP1, and anti-neuropilin-1 antisera using the corresponding immobilized peptide.

Please replace the paragraph at page 23, line 20 through page 24, line 18 with the following amended paragraph:

RT-PCR analysis. Total cellular RNAs were extracted from the purified oligodendrocytes using RNA-zol B (Bioprobe, Montreuil sous Bois, France), according to the manufacturer's instructions. Reverse transcription was performed on 1 µg of total RNA using oligo-dT primers and Moloney murine leukemia virus reverse transcriptase (Life Technology, Cergy Pontoise, France). Ten percent of the RT product was used to perform the PCR. The following pairs of synthetic oligonucleotides were used as primers : 5'-ATAGACACGATGCCAAGACCTTACC-3 (SEQ ID ~~n°~~NO: 16) and 5'-ATTACCGCACCATCCTCAAGGC-3' (SEQ ID ~~n°~~NO: 17) for CRMP1/Ulip3 (270 bp amplified cDNA fragment), 5'-T ATCACCCATCCCTTACTCTTCTGG-3' (SEQ ID ~~n°~~NO: 18) and 5'-CAGAAGAAAAAGCCAGAACAGACCG-3' (SEQ ID ~~n°~~NO: 19) for CRMP2/Ulip2 (141 bp amplified cDNA fragment), 5'-CCCCTCCCCATAAACTCTCTTTTGG-3' (SEQ ID ~~n°~~NO: 20) and 5'-CTGGAAAGTTCACAGGCTGG-3' (SEQ ID ~~n°~~NO: 21) for CRMP3/Ulip4 (200 bp amplified cDNA fragment), 5'-CCTACCAGGGCAAGAAGAACATTCC-3' (SEQ ID ~~n°~~NO: 22) and 5'-CCGCAATGGTCTTCACACCTCC-3' (SEQ ID ~~n°~~NO: 23) for CRMP4/Ulip1 (173 bp amplified cDNA fragment), 5'-CTGTGGATGTGGACATGAAGC-3' (SEQ ID ~~n°~~NO: 24) and 5'-AGCAATAAAC AGGTGGAAGGTC-3' (SEQ ID ~~n°~~NO: 25) for proteolipid protein (PLP) an oligodendrocytic marker, (Monge et al., 1986), 5'-AGAGAGATTCGCACTCA-3' (SEQ ID ~~n°~~NO: 26) and 5'-AGTGCCTCCTGGTAACTGG-3' (SEQ ID ~~n°~~NO: 27) for glial fibrillary associated protein (GFAP), an astrocytic marker (Palfreyman et al., 1979), and 5'-GAAGAGTGGTTCAAGAGCCG-3' (SEQ ID ~~n°~~NO: 28) and 5'-TGCCATCTTGACATTGAGGAGGTCC-3' (SEQ ID ~~n°~~NO: 29) for the low

molecular weight neurofilament protein (NF-L), a neuronal marker (Julien et al., 1987). The cDNA was denatured at 94°C for 5 minutes and then 35 cycles of PCR were carried out using Ampli-Taq DNA polymerase (Life Technology). The cycle profile consisted of denaturation at 94°C for 45 s, annealing at 62°C for 45 s, and elongation at 72°C for 2 min. The PCR products were fractionated by electrophoresis on 1.8 agarose gels. Cyclophilin cDNA was used as an internal control (Danielson et al., 1988). The specificity of the assay was checked by sequencing the RT-PCR amplified fragments.

Please replace the paragraph at page 26, lines 9-23 with the following amended paragraph:

In-situ hybridization. Sense or antisense digoxigenin-labeled riboprobes were generated by transcription of mouse Ulip2/CRMP2 cDNA (access number Y10339) and human Ulip6/CRMP5 cDNA (SEQ ID ~~N°~~NO: 1) in pBluescript SK, using the T3 or T7 promoters and labeling with digoxigenin-UTP (Roche, Meylan, France), following the manufacturer's instructions. The human Ulip6/CRMP5 cDNA-derived riboprobe was suitable for hybridization with rat tissue sections because the sequence of this human riboprobe displays more than 90 % homology with the corresponding rat sequence. Tissue sections were prepared as described above for immunohistochemistry, then treated with the sense and antisense riboprobes. For neuropilin-1, after 48 h of culture, purified oligodendrocytes were fixed in 4% paraformaldehyde, then subjected to in situ hybridization with digoxigenin-labeled oligonucleotide probes (antisense: CAGACATGTGATACCAGAAGGTCATGCAGT, SEQ ID ~~n°~~NO: 30, from the neuropilin-1 sequence, access number D50086) as described previously (Giger et al., 1996).

Please replace the paragraph at page 28, lines 4-25 with the following amended paragraph:

A human spinal cord cDNA library was screened using an anti-CV2 serum from a patient with PND and small cell lung carcinoma that recognized a 66 kDa protein on Western-blots of new-born rat brain protein extracts, but did not recognize any of the

four previously known Ulip/CRMP recombinant proteins. This led to the identification of one partial-length clone (C97) containing a 1.6 kb cDNA insert yielding a 90 amino acid open reading frame which showed 35% homology with the C-terminal region of the four known human Ulip/CRMP proteins. The cDNA containing the full-length coding region was obtained by screening the same library with a radioactive probe corresponding to the coding region of C97 (270 bp). A 2 kb cDNA, referred to as Ulip6/CRMP5, that contains an open reading frame coding for 564 amino acids, was isolated (SEQ ID ~~n°~~NO: 1). The C-terminal region of this protein was identical to the 90 amino acids encoded by C97. On Western blots, the Ulip6/CRMP5 recombinant protein was recognized by all 20 anti-CV2 sera tested (Fig. 1B), but not by 100 sera from patients without PND (half of them having small cell lung carcinoma), suggesting that Ulip6/CRMP5 was the major antigen recognized by anti-CV2 antibodies. The overall sequence of the Ulip6/CRMP5 cDNA consists of 3074 bp made up of a 162 bp 5'-non-coding region, a 1692 bp protein coding region, and a 1220 bp 3'-non-coding region. The initiation codon was assigned to the Met codon at position 163-165. The deduced protein sequence predicted a protein with a molecular mass of 61.424 kDa and an isoelectric point of 7.46.